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Computation of kinetic isotope effects for enzymatic reactions

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Abstract

We describe a computational approach, incorporating quantum mechanics into enzyme kinetics modeling with a special emphasis on computation of kinetic isotope effects. Two aspects are highlighted: (1) the potential energy surface is represented by a combined quantum mechanical and molecular mechanical (QM/MM) potential in which the bond forming and breaking processes are modeled by electronic structure theory, and (2) a free energy perturbation method in path integral simulation is used to determine both kinetic isotope effects (KIEs). In this approach, which is called the PI-FEP/UM method, a light (heavy) isotope is mutated into a heavy (light) counterpart in centroid path integral simulations. The method is illustrated in the study of primary and secondary KIEs in two enzyme systems. In the case of nitroalkane oxidase, the enzymatic reaction exhibits enhanced quantum tunneling over that of the uncatalyzed process in water. In the dopa decarboxylase reaction, there appears to be distinguishable primary carbon-13 and secondary deuterium KIEs when the internal proton tautomerism is in the N-protonated or in the O-protonated positions. These examples show that the incorporation of quantum mechanical effects in enzyme kinetics modeling offers an opportunity to accurately and reliably model the mechanisms and free energies of enzymatic reactions.

Keywords

combined QM/MM; path integral simulations; PI-FEP/UM; kinetic isotope effects; enzyme kinetics

1 Introduction

A major goal in molecular biology is the elucidation of the origin of the remarkable power of enzymes to enhance the rate of chemical reactions [1]. Although it is clear that the dominant factor in enzyme catalysis is the lowering of the free energy barrier of an enzymatic reaction in comparison with the uncatalyzed process [1–3], an intriguing and controversial question is whether enzymes have evolved to enhance tunneling to accelerate the reaction rate [4]. This is particularly critical for reactions involving hydrogen (proton, hydride and hydrogen atom) transfer — one of the most prevalent processes in biology — because of its relatively small mass, which possesses the greatest quantum effects [5–7]. All reactions involve some degrees of quantum effects, which include both zero-point energy [8] and tunneling [7], and it is necessary to incorporate these effects into the theory to compute reaction rates [7–9]. The controversy lies in the notion whether enzymes have evolved to fine-tune catalysis such that there is greater tunneling in the enzyme than the uncatalyzed reaction in water. In principle, this question can be addressed by comparing the enzymatic and the uncatalyzed reaction in solution [10–11]; however, the difficulty is to design a

model system that mimics exactly the same enzymatic reaction and mechanism. Furthermore, quantum effects on rate acceleration, if any, are expected to be much smaller than other factors such as hydrogen bonding and the overall stabilization of the transition state [1, 3, 12]. Consequently, some researchers simply dismiss the significance of tunneling in catalysis. Nevertheless, a factor of two in rate enhancement can still have an important physiological impact. Quantum tunneling cannot be measured directly. The most direct experimental probe to transition state and tunneling of an enzymatic reaction is through measurements of kinetic isotope effects (KIE) [5, 13], and the interpretation of the experimental data requires the aid of theoretical analyses. In this article, we review a path integral computational method that has been developed in our laboratory to accurately determine both the primary (a relatively easy problem if hydrogen is involved) and secondary (a more challenging quantity and critical test of computational methods) KIEs for enzymatic reactions. The method is illustrated by applications to nitroalkane oxidase and L-dopa decarboxylase.

There are two main components necessary to incorporate quantum mechanics in enzyme kinetics modeling [7, 14]. First, electronic structural theory is required to provide an accurate representation of the potential energy surface (PES) and the description of the transition structure. The most successful approach is to treat the enzyme active site with electronic structure theory and the remainder of the system by molecular mechanics. Such a combination of quantum mechanics with molecular mechanics (QM/MM) offers the accuracy of a quantum chemical representation of the part of the system of primary interest, which can be systematically improved, and computational efficiency of a classical force field, which allows for dynamics simulations and conformational sampling to be carried out. To further increase the capability and reliability of biomolecular computation, it is likely that a full quantum mechanical representation of the entire enzyme system in solution is required, which explicitly incorporates electronic polarization and charge transfer effects [15]. The second component is to incorporate nuclear quantum effects (NQE) into the time-evolution of macromolecular systems, including zero-point energy, non-adiabatic coupling and quantum mechanical tunneling. The NQEs are essential in the study of hydrogen transfer processes and the computation of KIEs [7].

In this article, we describe a novel computational approach for estimating kinetic isotope effects in enzymatic reactions using free energy perturbation theory on isotopic masses in Feynman path integral simulations. The use of a combined QM/MM potential is first summarized, which is followed by a description of centroid path integral simulation in quantum transition state theory. The method is highlighted in two specific applications, which shows, respectively, that there is enhanced tunneling in the enzymatic process catalyzed by nitroalkane oxidase, and that the computational results in the dopa decarboxylase reaction can be used to assess the catalytic mechanism and cofactor tautomeric equilibrium in the active site.

2 Method

2.1 Combined QM/MM potential

The accuracy of the potential energy function used to carry out molecular dynamics simulations of an enzymatic process directly affects the reliability of the computational results [1, 14, 16–17]. In our study of chemical and enzymatic reactions, the potential energy surface (PES) is constructed using QM/MM methods, in which the active site is treated explicitly by electronic structure theory and the remainder of the system is approximated by classical force fields [16, 18–19]. The QM/MM potential is given by

$$U_{tot} = \langle \Psi(\mathbf{R}) | H_{qm}^o(\mathbf{R}) + H_{qm/mm}(\mathbf{R}) | \Psi(\mathbf{R}) \rangle + U_{mm} \quad (1)$$

where $H_{qm}^o(\mathbf{R})$ is the Hamiltonian of the QM-subsystem $\mathbf{R}$, U_{mm} is the MM potential energy for the rest of the system, and $H_{qm/mm}(\mathbf{R})$ is the interaction Hamiltonian between the two regions. The wave function $\Psi(\mathbf{R})$ of the QM-subsystem at a given nuclear position $\mathbf{R}$ is polarized by the external potential $H_{qm/mm}(\mathbf{R})$.

It is most convenient to rewrite eq. (1) as follows [18]:

$$U_{tot} = E_{qm}^o(\mathbf{R}) + \Delta E_{qm/mm}(\mathbf{R}) + U_{mm} \quad (2)$$

where $E_{qm}^o(\mathbf{R})$ is the energy of an isolated QM-subsystem,

$$E_{qm}^o(\mathbf{R}) = \langle \Psi^o(\mathbf{R}) | H_{qm}^o(\mathbf{R}) | \Psi^o(\mathbf{R}) \rangle \quad (3)$$

The interaction energy in eq. (2), $\Delta E_{qm/mm}(\mathbf{R})$, corresponds to the energy change of transferring the QM subsystem from the gas phase into the enzyme active site:

$$\Delta E_{qm/mm}(\mathbf{R}) = \langle \Psi(\mathbf{R}) | H_{qm}^o(\mathbf{R}) + H_{qm/mm}(\mathbf{R}) | \Psi(\mathbf{R}) \rangle - E_{qm}^o(\mathbf{R}) \quad (4)$$

We note that eq. (2) is especially useful because the total energy of a hybrid QM and MM system is separated into two “independent” terms – the gas-phase energy and the interaction energy – which can now be evaluated using different methods or at different levels of theory. Consequently, we can use a high-level (*HL*) theory to describe the internal energies of the QM system ($E_{qm}^o(\mathbf{R}) = E_{qm}^{HL}(\mathbf{R})$), and a low-level (*LL*) method to carry out molecular dynamics simulations to determine the QM-MM interaction term

($\Delta E_{qm/mm}(\mathbf{R}) = \Delta E_{qm/mm}^{LL}(\mathbf{R})$). This approach, which is called a dual-level (*DL*) method, has been used by our group and by Tunon and Moliner and coworkers [20]:

$$U_{tot}^{DL}(\mathbf{R}) = E_{qm}^{HL}(\mathbf{R}) + \Delta E_{qm/mm}^{LL}(\mathbf{R}) + U_{mm} \quad (5)$$

Usually, the semiempirical Austin Model 1 (AM1), parameterization model 3 (PM3), or other semiempirical approaches such as the tight-binding density functional theory (DFTB) model are used in connection with a force field. We have used the standard CHARMM22 force field in our applications. The higher-level method is chosen on the basis of the size of the QM subsystem and the accuracy of the QM model for a particular system. Typically, at least MP2 or G2 quality of method or a tested DFT model is used with a large basis set.

The term $\Delta E_{qm/mm}(\mathbf{R})$ in a combined QM/MM potential contains empirical parameters to account for short-range repulsion and dispersion interactions, which should be optimized to accurately describe QM/MM interactions. By systematically optimizing the associated van der Waals parameters for the “QM-atoms”, both semiempirical and *ab initio* (Hartree-Fock) QM/MM potentials can yield excellent results for hydrogen-bonding and dispersion interactions in comparison with experimental data [21]. We have validated QM/MM performance through extensive studies of solvation free energies, polarization effects, the potentials of mean force for organic reactions, and the effects of solvation on molecular structures and on electronic transitions.

2.2 Quantum transition state theory

2.2.1 Centroid path integral simulations—In the discrete Feynman path integral (PI) method, each quantized nucleus is represented by a ring of P quasiparticles, whose coordinates are denoted as $\mathbf{r}_i^{(n)} = \{\mathbf{r}_i^{(n)}; i=1, \dots, P\}$ where n is the n th quantized atom [22]. The discrete paths are circular, in which each particle (bead) is connected with its neighbors by a harmonic potential with a force constant of $k^{(n)} = 2\pi P / \beta \lambda_n^2$, where $\lambda_n = (2\pi\beta\hbar^2 / M_n)^{1/2}$ is the de Broglie thermal wavelength of the atom with a mass of M_n and $\beta = 1/k_B T$ with k_B being Boltzmann's constant and T being the temperature. The canonical QM partition function of a hybrid quantum and classical system, consisting of one quantized atom (for convenience of discussion), can be written as follows [22].

$$Q_p^{qm} = \frac{1}{\Omega} \int d\mathbf{S} \int d\mathbf{s} \left(\frac{P}{\lambda_M^2} \right)^{3P/2} \int d\mathbf{R} e^{-\beta V_{eff}(\{\mathbf{r}\}, \mathbf{S})} \quad (6)$$

where Ω is the volume element of classical particles, $\mathbf{S}$ represents the coordinates of all classical (solvent) particles, $\mathbf{s}$ denotes the coordinates of the quantized particle, and $\int d\mathbf{R} = \int d\mathbf{r}_1 \cdots \int d\mathbf{r}_p \delta(\bar{\mathbf{r}} = \mathbf{s})$, where $\delta(\bar{\mathbf{r}} = \mathbf{s})$ indicates that the average coordinates of the P quasiparticles is the same as the classical coordinates of the quantized atom. The effective potential $V_{eff}(\{\mathbf{r}\}, \mathbf{S})$ is given by

$$V_{eff}[\{\mathbf{r}\}, \mathbf{S}] = \frac{\pi P}{\beta \lambda_M^2} \sum_i^P (\mathbf{r}_i^{(1)} - \mathbf{r}_{i+1}^{(1)})^2 + \frac{1}{P} \sum_i^P U(\mathbf{r}_i^{(1)}, \mathbf{S}) \quad (7)$$

where the superscript (1) specifies the single quantized atom (which can be easily generalized to N quantized atoms), and $U(\mathbf{r}_i^{(1)}, \mathbf{S})$ is the potential energy of the system for the i th quasiparticle.

In centroid path integral, the centroid position, $\bar{\mathbf{r}}$, is used as the principle variable, defined as the geometrical average of the quasiparticles [23–28]:

$$\bar{\mathbf{r}}^{(n)} = \frac{1}{P} \sum_{i=1}^P \mathbf{r}_i^{(n)} \quad (8)$$

where the superscript (n) specifies the n th quantized atom. The parameter P is chosen to be sufficiently large such that the numerical result converges to the quantum limit.

With the correspondence of classical coordinates and centroid positions, one can define an effective quantum mechanical potential of mean force (PMF) $w(z)$ as a function of the centroid reaction coordinate z by [29]

$$e^{-\beta w(z)} = \frac{\langle \partial(z[\bar{\mathbf{r}}] - z) \rangle}{\langle \delta(z[\bar{\mathbf{r}}] - z_R) \rangle} \quad (9)$$

where z_R is the minimum point at the reactant state in the PMF and the ensemble average $\langle \cdots \rangle$ is obtained by the effective potential of eq. (7). However, the dynamics generated by the effective potential of eq. (7) has no physical significance, and it is used purely as a procedure to obtain the correct integral [22, 30]. Note that a PMF as a function of finite reaction coordinate is at odds with quantum mechanical nature of the system. Nevertheless, the reaction coordinate function $z[\bar{\mathbf{r}}]$ can be evaluated from the path centroids $\bar{\mathbf{r}}$ [28, 31–33], first recognized by Feynman and Hibbs as the most classical-like variable in quantum statistical mechanics [22].

In practice, it is useful to rewrite eq. (6) as a double average [22, 33–35]:

$$\frac{Q_{qm}}{Q_{cm}} = e^{-\beta \Delta \Delta w_{cm \rightarrow qm}} = \ll e^{-\beta \Delta \bar{U}(\bar{\mathbf{r}}, \mathbf{S})} \gg_{FP, \bar{\mathbf{r}}} \gg_U \quad (10)$$

where the factor Q_{cm} is the classical partition function defined in [22], $\Delta \Delta w_{cm \rightarrow qm}$ is the free energy difference between the quantized and classical systems, and the average $\langle \cdots \rangle_U$ is a purely classical ensemble average obtained according the potential $U(\mathbf{r}, \mathbf{S})$, evaluated using the centroid coordinates. In eq. (10), the average differential potential is given by

$$\Delta \bar{U}(\bar{\mathbf{r}}, \mathbf{S}) = \frac{1}{P} \sum_i^P \{U(\mathbf{r}_i, \mathbf{S}) - U(\bar{\mathbf{r}}, \mathbf{S})\} \quad (11)$$

In eq. (11), the potential $U(\mathbf{r}_i, \mathbf{S})$ is determined at the coordinates of the i th quasiparticle for the quantized atom, and a total of P separate, individual QM/MM computations are needed for each configuration in the path integral sampling. The inner average $\langle \cdots \rangle_{FP, \bar{\mathbf{r}}}$ in eq. (10) represents a path-integral free-particle sampling, carried out without the external potential $U(\mathbf{r}, \mathbf{S})$ [22, 33, 35].

The double averaging procedure (eq. (10)) is the theoretical basis of the present centroid path integral simulation, and it was used by Sprik *et al.*, called the hybrid classical and path integral [35], and by Hwang and Warshel, called QCP [33, 36]. The double average of eq. (10) yields the exact path integral centroid density, which can be used to determine the centroid PMF [33, 37]:

$$e^{-\beta w(\bar{\mathbf{z}})} = e^{-\beta w_{cm}(\bar{\mathbf{z}})} e^{-\beta \Delta \Delta w_{cm \rightarrow qm}(\bar{\mathbf{z}})} \quad (12)$$

where $w(\bar{\mathbf{z}})$ and $w_{cm}(\bar{\mathbf{z}})$ are the centroid quantum mechanical and the classical mechanical PMF. Eq. (12) is particularly useful because the quantum mechanical PMF can be evaluated in two simulation steps, first by carrying out a classical mechanical molecular dynamics simulation, followed by free-particle path integral sampling with the constraint that the centroid coordinates of the quantized atoms coincide with the corresponding classical positions (eq. (10)) from the trajectory generated in the first step.

To enhance convergence of free-particle sampling in centroid path integral simulations, we have developed a bisection sampling technique for a ring of beads, by extending the original approach of Ceperley for free particle sampling in which the initial and final beads are not connected [38–39]. Since the free-particle distribution is known exactly at a given temperature, each ring-beads distribution is generated according to this distribution and thus 100% accepted [39]. Furthermore, in this construction, each new configuration is created independently, starting from a single initial bead position, allowing the new configuration to move into a completely different region of configurational space. This latter point is especially important in achieving convergence by avoiding being trapped in a local region of the classical potential in the path integral sampling. This method has been thoroughly tested in a number of applications [37, 40–43].

2.2.2 Quantum transition state theory—The theoretical framework for enzyme kinetics modeling is path integral quantum transition state theory (QTST) [24, 28, 32], which was derived by writing the rate expression analogous to classical transition state theory:

$$k_{\text{QTST}} = \frac{1}{2} \langle |\dot{z}| \rangle_{z^\ddagger} e^{-\beta w(z^\ddagger)} / \int_{-\infty}^{z^\ddagger} dz e^{-\beta w(z)} \quad (13)$$

where $w(z)$ is the quantum mechanical PMF as a function of the centroid reaction coordinate $z[\mathbf{r}]$, z is the value of $z[\mathbf{r}]$ at the maximum of the PMF relative to the minimum at the reactant state, and $\langle |\dot{z}| \rangle_{z^\ddagger} = (2/\pi\beta M_{\text{eff}})^{1/2}$ is a dynamical frequency factor approximated by the velocity for a free particle of effective mass M_{eff} along the reaction coordinate direction.

The exact rate constant is obtained by multiplying the QTST rate constant by a correction factor or transmission coefficient γ_q [32]:

$$k = \gamma_q \cdot k_{\text{QTST}} \quad (14)$$

Although eqs. (13) and (14) have identical forms to that of the classical rate constant, unlike classical variational transition state theory, there is no variational upper bound in the QTST rate constant because the quantum transmission coefficient γ_q may be either greater than or less than one [28].

There is no practical procedure to compute the quantum transmission coefficient γ_q in eq. (14). For a model reaction with a parabolic barrier along the reaction coordinate coupled to a bath of harmonic oscillators, the quantum transmission coefficient is the Grote-Hynes (GH) classical transmission coefficient κ_{GH} [24, 44]. Often, the classical γ_q is used to approximate the quantum transmission coefficient; however, there is no correspondence between classical and quantum dynamic trajectories and the effects of tunneling may greatly affect reaction dynamics near the barrier top. Studies have shown that the activation free energy in the centroid PI-QTST “captures most of the tunneling and quantization effects”; which give rise to deviations from classical TST [45–48].

2.3 The PI-FEP/UM method for accurate computation of KIEs

The centroid path integral method enables us to conveniently determine kinetic isotope effects by directly computing the ratio of the quantum partition functions for two different isotopes through free energy perturbation (FEP) theory. In essence, only one simulation of a given isotopic reaction, e.g., the light isotope, is performed, while the ratio of the partition function, i.e., the KIE, to a different isotopic reaction, is obtained through FEP by perturbing the mass from the light isotope to the heavy isotope, along the PMF obtained using umbrella sampling (UM) [37]. This is in contrast to other approaches that have been reported in the literature employing centroid path integral simulations [33, 49, 50], in which two separate simulations are performed. The use of “mass” perturbation in free-particle bisection sampling scheme results in a major improvement in computation accuracy for KIE calculations such that secondary kinetic isotope effects and heavy atom isotope effects can be reliably obtained [29, 37, 51].

The light-to-heavy kinetic isotope effect is written in terms of the ratio of the partial partition functions at the centroid reactant and transition state and is given by [37]:

$$\text{KIE} = \frac{k^L}{k^H} = \left[\frac{Q_{qm}^L(\bar{z}_L^\ddagger)}{Q_{qm}^H(\bar{z}_L^\ddagger)} \right] \left[\frac{Q_{qm}^H(\bar{z}_H^R)}{Q_{qm}^L(\bar{z}_L^R)} \right] \times \exp \left(-\beta \{ w_L^R(\bar{z}_L^R) - w_H^R(\bar{z}_H^R) \} \right) \quad (15)$$

where $\bar{z}_{L/H}^\ddagger$ and $\bar{z}_{L/H}^R$ specify the reaction coordinate values at the transition state and reactant state for light (L) and heavy (H) isotopes, respectively. The ratio of the partition function in eq. (15) can be written as follows:

$$\frac{Q_{qm}^H(\bar{z})}{Q_{qm}^L(\bar{z})} = \frac{\langle \delta(\bar{z} - \bar{z}) \langle e^{-\frac{\beta}{P} \sum_i \Delta U_i^{L \rightarrow H}} e^{-\beta \Delta \bar{U}_L} \rangle_{FP,L} \rangle_U}{\langle \delta(\bar{z} - \bar{z}) e^{-\beta [F_L(\bar{z}, S) - F_{FP}^o]} \rangle_U} \quad (16)$$

where the subscripts L specifies that the ensemble averages are done using the light isotope, $\Delta \bar{U}_L$ is defined by eq. (11), F_{FP}^o is the free energy of the free particle reference state for the quantized particles [22], and $\Delta U_i^{L \rightarrow H} = U(\mathbf{r}_{i,H}) - U(\mathbf{r}_{i,L})$ represents the difference in “classical” potential energy at the heavy and light bead positions $\mathbf{r}_{i,H}$ and $\mathbf{r}_{i,L}$.

In the bisection sampling scheme [40], the perturbed heavy isotope positions are related to the lighter ones by

$$\frac{\mathbf{r}_{i,L}}{\mathbf{r}_{i,H}} = \frac{\lambda_{M_L} \boldsymbol{\theta}_i}{\lambda_{M_H} \boldsymbol{\theta}_i} = \sqrt{\frac{M_H}{M_L}}; \quad i=1, 2, \dots, P \quad (17)$$

where $\mathbf{r}_{i,L}$ and $\mathbf{r}_{i,H}$ are the coordinates for bead i of the corresponding light and heavy isotopes, λ_{M_L} and λ_{M_H} are isotopic masses for the light and heavy nuclei, and $\boldsymbol{\theta}_i$ is the position vector in the bisection sampling scheme which depends on the previous sequence of directions and has been fully described in reference [40].

In eq. (16), we obtain the free energy (inner average) difference between the heavy and light isotopes by carrying out the bisection free-particle sampling with the light atom and then perturbing the heavy isotope positions according to eq. (17). Then, the free energy difference between the light and heavy isotope ensembles is weighted by a Boltzmann factor for each quantized configuration [37].

3 Illustrative examples

3.1 Nitroalkane oxidase

Nitroalkane oxidase (NAO), which consists of a flavin adenine dinucleotide (FAD) cofactor in the active site, catalyzes the conversion of nitroalkanes to nitrite and aldehydes or ketones [52]. The initial α -proton abstraction of the small substrate nitroethane by Asp402 is rate-limiting (Figure 1). Because of the simplicity of the chemical step, a model uncatalyzed reaction between nitroethane and acetate ion in aqueous solution has been used experimentally to assess the enzyme activity. Valley *et al.* found that NAO accelerates the proton abstraction reaction by a factor of 10^9 over the uncatalyzed process in water [11], and that the deuterium kinetic isotope effects (KIEs) are noticeably greater for the enzymatic reaction (9.2) than that in water (7.8) [11, 53]. The latter suggesting that tunneling contribution may play a greater role in the enzyme than that in water; however, the small difference between the enzymatic and uncatalyzed KIEs is not sufficient to make a definitive conclusion. Computational studies offer an opportunity to help interpret the experimental data and to provide further insight into quantum tunneling in the enzymatic process.

We used the PI-FEP/UM simulation technique to estimate the NQEs [37], both for the NAO-catalyzed and the uncatalyzed proton abstraction of nitroethane by acetate ion [51]. In this study, the PES was described by a combined QM/MM potential [18, 42], in which a set of specific reaction parameters (SRP) has been developed within the AM1 formalism to fit results from high-level *ab initio* theory as well as from experiments [37, 42]. The crystal structure (PDB id: 2C12) with the inhibitor spermine was used to construct the Michaelis complex (MC) model through a combined minimization and molecular dynamics simulation technique [54]. Later, it was shown that the computational MC structure is in excellent accord with a parallel study of the D402N-Nitroethane X-ray structure [51].

The centroid PMFs from PI-FEP/UM simulations for the nitroethane deprotonation reaction in NAO and in water (Scheme 1) are shown in Figure 2. The reaction coordinate is defined as the difference between the breaking (donor-proton) and forming (acceptor-proton) bond distances (Scheme 1). In both systems, the centroid PMFs were determined by quantizing the primary proton that is being abstracted as well as the donor and acceptor atoms (Figure 3), whereas in the secondary KIE calculations, the secondary proton of the methylene group of nitroethane is also quantized. The computed free energies of activation are 15.9 and 24.4 kcal/mol for the enzymatic and the uncatalyzed proton transfer reaction in water, respectively, in accord with experimental results (14.0 and 24.8 kcal/mol) [11].

The computed primary and secondary KIEs for the nitroethane deprotonation reaction in water and in the enzyme are listed in Table 1 along with the total KIEs that have been determined for the perdeuterated substrate nitroethane at the C_α position [11]. In PI-FEP/UM simulations, the computed nuclear quantum effects include both zero-point energies and nuclear tunneling and their contributions to the computed KIEs are not separable [37]. Thus, it is useful to use semiclassical methods [55] to estimate the tunneling transmission coefficient to gain an understanding of the origin of NQEs in NAO catalysis. Consequently, we have utilized the ensemble averaged variational transition state theory with multidimensional tunneling (EA-VTST/MT) approach that has been developed earlier [7, 14, 56] to separate tunneling from the overall quantum effects (Table 1).

The results in Figure 2 and Table 1 present strong evidence that nuclear quantum effects make greater contributions to the enzymatic reaction in nitroalkane oxidase than the uncatalyzed process in water. First, there are greater NQEs to lower the classical barrier of the enzymatic process than that of the uncatalyzed model reaction. Second, the difference in quantum effects between proton and deuteron transfer is more significant for the enzymatic reaction than the uncatalyzed one. Third, both experiment and computation show enhanced primary KIEs in the enzyme over that in water. Fourth, the mixed Swain-Schaad exponent for the enzymatic reaction is greater than the semiclassical limit without tunneling and it is increased for the enzyme process. Finally, the tunneling transmission coefficient was found to be about three times greater for the enzyme reaction than the model reaction in water from EA-VTST/MT analysis, an entirely different approach from the PI-FEP/UM simulation method. Analyses of the tunneling paths in EA-VTST (details can be found in ref. [51]) reveal that the origin of the difference may be attributed to a narrowing effect in the effective potentials for tunneling in the enzyme than that in aqueous solution [51]. These studies demonstrate that differential quantum tunneling contributions are utilized in certain enzymatic catalysis as illustrated by nitroalkane oxidase.

3.2 Decarboxylation and kinetic isotope effects in dopa decarboxylase

The enzyme L-dopa decarboxylase (DDC) [57] catalyzes the conversion of L-dopa into dopamine in the treatment of Parkinson's disease [58, 59]. DDC is a Pyridoxal 5'-phosphate (PLP) dependent enzyme, in which the carboxylate side chain of Asp271 accepts a hydrogen bond from the protonated pyridinium ion of the cofactor PLP, a typical but not unique feature in PLP-dependent enzymes [57]. In the absence of substrate, PLP is covalently linked to a lysine residue in the active site, forming a so-called internal aldimine complex. When an amino acid substrate is bound, the protonated Schiff base linkage is exchanged with the amino group of the substrate, producing an external aldimine binary complex. The proton that forms an internal hydrogen bond between the imine nitrogen and the 3-oxo anion of the external aldimine between PLP and the amino acid substrate can undergo an intramolecular proton transfer from the N-protonated Schiff base to the O-protonated hydroxyimine, formally corresponding to a tautomerization equilibrium (Scheme 2). This equilibrium plays a role that affects the reactivity of the PLP Schiff base in the active site. Richard and coworkers suggested that PLP-dependent enzymes select the

zwitterionic external aldimine complex, in which pronation at the imine nitrogen is preferred [60, 61].

We used the semiempirical AM1 Hamiltonian without further modification to model the decarboxylation reaction of the external aldimine substrate PLP(H⁺)-L-dopa, which consists of a total of 39 atoms (Scheme 3) [62]. Previous studies of several decarboxylation reactions [41, 63–67] have shown that the AM1 model can yield good geometrical and energetic results for the decarboxylation reaction. Figure 4 shows the computed PMFs, including NQE, for the DDC-catalyzed and the corresponding uncatalyzed PLP-dopa external aldimine in water [68]. In aqueous solution, the free energy barriers for the decarboxylation reaction of the PLP-dopa external aldimine complex are essentially the same both for the zwitterionic PSB and the neutral O-protonated tautomers, suggesting that there is no particular preference for the α -carbanion stabilization by a neighboring (N-protonated) iminium ion in the dopa decarboxylation reaction. The enzyme lowers the free energy barrier by about 6 kcal/mol with the PLP cofactor in the N-protonated PSB and by 8 kcal/mol in O-protonated PLP configuration. The computed barrier height is 17.3 kcal/mol including NQEs, which is in accord with the experimental value of 16.0 kcal/mol [59]. The uncatalyzed decarboxylation of the external aldimine has a barrier of 42 kcal/mol in water, consistent with Wolfenden's experimental data on similar systems (41 kcal/mol) [69]. Thus, the PLP cofactor itself provides a dominant contribution to the overall catalytic effects [61], lowering the decarboxylation barrier by about 16 kcal/mol. The enzyme further lowers the barrier by another 8 kcal/mol.

Listed in Table 2 are the computed 1° and 2° KIEs from PI-FEP/UM simulations along with key geometrical results at the transition state. In DDC, we obtained values of 1.053 and 1.047, respectively, for the reaction of the O-protonated and the N-protonated isomer of the external aldimine [68]. These fall into the range of KIEs measured for similar reactions [70, 71]. The calculated carboxyl ¹³C-KIEs in Table 2 are closely related to the variations in the O–C–O bond angle of the carboxylate group. The transition state of the O-protonated tautomer at the active site has the smallest O–C–O bond angle of the four decarboxylation reactions, indicating an early transition state with the least gain in bond order on the CO₂ group. On the other hand, the N-protonated isomer has the largest O–C–O angle, indicative of enhanced force constant in the CO₂ stretch and a late transition state (mirrored by a larger value in the reaction coordinate). Thus, for the N-protonated configuration of the PLP cofactor in the enzymatic reaction, the reduction in the difference of zero-point energy (ZPE) (between ¹²C and ¹³C) associated with the C_α-C stretch is accompanied by an increase in the O–C–O stretching frequency, orthogonal to the reaction coordinate, in going from the reactant to the transition state. The two compensating factors yield a smaller computed carboxyl KIE in the N-protonated PLP aldimine substrate. The change in O–C–O angle is less for the O-protonated configuration, resulting in a greater KIE at the carboxyl site of the substrate.

The rehybridization at the C_α position from sp³ to sp² along the decarboxylation path is best reflected by the 2° ¹k/²k (H_a) KIEs (Table 2). The development of the C_α=N double bond character is indicated by the distance R(C_α=N) in Table 2. In the N-protonated configuration (a longer C_α=N bond distance at the transition state), the dominant factor is the change in the C–H deformation mode, giving rise to greater decrease in ZPE, thereby, relatively large secondary KIEs. For the O-protonated isomer of the cofactor, a greater C_α=N double bond character is developed, resulting in an increase in the C–H stretch at the transition state. Thus, the 2° ¹k/²k (H_a) KIEs are relatively small and even inverse for the reaction in water (Table 2).

4 Conclusions

In this article, we summarize an approach that incorporates quantum mechanics into enzyme kinetics model with a special focus on centroid path integral simulation of kinetic isotope effects. Two aspects are emphasized: (1) the potential energy surface is represented by a combined quantum mechanical and molecular mechanical (QM/MM) potential in the bond forming and breaking processes are modeled by electronic structure theory, and (2) a free energy perturbation approach to determine both primary and secondary kinetic isotope effects (KIEs) in which a light (heavy) isotope is mutated into an heavy (light) counterpart in Feynman centroid path integral simulations. The latter method is called the PI-FEP/UM approach, which is illustrated in calculations of the primary and secondary KIEs in the proton abstraction of nitroethane by an active site aspartic acid, and heavy atom (and deuterium) KIEs involved in the decarboxylation reaction of L-dopa by dopa decarboxylase. We found that in the case of NAO, the enzymatic reaction exhibits enhanced quantum tunneling over that of the uncatalyzed process in water. Although the computed tunneling enhancement is relatively small (by a factor of about 3) relative to the effect of transition state stabilization (by a factor 10^8), the presence of nuclear quantum effects in rate acceleration is clear and the magnitude can still have significant physiological consequences. In the DDC decarboxylation reaction, there appears to be distinguishable primary carbon-13 and secondary deuterium KIEs when the internal proton tautomerism is in the N-protonated or in the O-protonated positions, and it would be interesting to validate these computational results through experimental measurements on this enzyme. These examples show that the incorporation of quantum mechanical effects in enzyme kinetics calculations offers a great opportunity to more accurately and reliably model the mechanism and free energies of enzymatic reactions.

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Biography

GAO JiaLi studied at Beijing University, and his professional career follows graduate work at Purdue, postdoctoral research at Harvard, and faculty positions at the State University of New York at Buffalo and the University of Minnesota. He is currently the Lee I. Smith Professor of Chemistry. His work focuses on the structure and properties of macromolecular systems. This includes the understanding of protein dynamics and enzyme catalysis, biomolecular interactions and assembly, and biophysical systems biology, as well as the development of quantum and classical mechanical methodologies.

Figure 1.

A snapshot of the active site of nitroalkane oxidase (NAO) corresponding to the proton abstraction product in which the basic residue Asp402 is already protonated. A key hydrogen bonding interaction between the nitro group of the substrate nitroethane and the cofactor flavin adenine dinucleotide. Image prepared by Dr. Dan Major.

Figure 2. Computed potentials of mean force with and without nuclear quantum effects for the proton (red) and deuteron (blue) transfer from nitroethane to acetate ion in water (dashed curves), and to Asp402 (solid curves) at the active site of nitroalkane oxidase. The classical mechanical potentials of mean force are given in green.

Figure 3.

A snapshot configuration of the path integral simulation of the proton abstraction of nitroethane by acetate ion in water. The proton being transferred and the donor and acceptor atoms are quantized with 32 quasiclassical particles in the simulation. Image prepared by Dr. Dan Major.

Figure 4. Computed potentials of mean force for the dopa decarboxylase-catalyzed (solid) and the uncatalyzed (dashed) decarboxylation of the PLP-dopa external aldimine in the N-protonated configuration in green and O-protonated tautomer in red.

Scheme 1.

Proton transfer from nitroethane to acetate (Asp402) ion.

Scheme 2.

Internal proton transfer tautomerism of the external aldimine of PLP cofactor and L-dopa, and the decarboxylation reaction catalyzed by L-dopa decarboxylase.

Scheme 3.

The L-dopa-PLP external aldimine. Atoms in blue are treated quantum mechanically and the rest of the cofactor and enzyme are represented by molecular mechanics. The boundary atom at the C5A position is modeled using the generalized hybrid orbital (GHO) method.

Table 1

Computed H/D, H/T and D/T kinetic isotope effects, and the overall computed and experimental deuterium isotope effects for the proton transfer from nitroethane to Asp402 in NAO and to acetate ion in water

1°	k_H^H/k_D^H	k_H^H/k_T^H	k_D^D/k_T^D
H ₂ O	6.63 ± 0.31	13.0 ± 1.0	2.17 ± 0.04
NAO	8.36 ± 0.58	18.1 ± 2.4	2.38 ± 0.05
2°	k_H^H/k_H^D	k_H^H/k_H^T	k_D^D/k_D^T
H ₂ O	1.340 ± 0.132	1.375 ± 0.183	1.096 ± 0.048
NAO	1.213 ± 0.150	1.229 ± 0.209	1.050 ± 0.025
k_H^H/k_D^D	Calc.	Expt. [11]	
H ₂ O	8.3 ± 1.1	7.8 ± 0.1	
NAO	10.1 ± 1.4	9.2 ± 0.4	

Subscripts and superscripts are used to specify the rate constant for isotope substitutions at the primary and secondary position, respectively.

Table 2

Computed kinetic isotope effects and selected bond lengths (angstroms) and angles (degrees) at the transition state

	Dopa decarboxylase		Aqueous solution	
	O-protonated	N-protonated	O-protonated	N-protonated
$^{12}k/^{13}k$ (C)	1.053 ± 0.002	1.047 ± 0.003	1.053 ± 0.003	1.049 ± 0.002
$^{12}k/^{13}k$ (C_a)	1.037 ± 0.006	1.026 ± 0.005	1.030 ± 0.003	1.034 ± 0.003
$^1k/^2k$ (H_a)	1.053 ± 0.032	1.144 ± 0.066	0.975 ± 0.051	1.137 ± 0.051
$R(C_a-C)$	2.07	2.17	2.06	2.17
$R(C_a=N)$	1.36	1.39	1.34	1.38
$\angle(O=C=O)$	148.6	153.8	150.5	153.2

Standard errors ($\pm 1\sigma$) in computed kinetic isotope effects are determined over ten separated block averages. Each KIE is obtained by using all data sampled in the PI-FEP/UM simulations